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PRINCIPAL INVESTIGATOR: YI ZHONG

CONTRACTING ORGANIZATION: Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724-2209

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6. AUTHOR(S)
YI ZHONG

E-Mail: zhongyi@cshl.edu

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14. ABSTRACT This three-year grant supports our efforts in studying a role of the neurofibromatosis type 1 (NF1) gene in mediating retrieval of long-term memory. Our investigation over the entire funding period leads to following discoveries. First, NF1 is critical for supporting multiple types of long-term memories, including aversive and appetitive classic conditioning. Second, it regulates memory bi-directionally. A loss of NF1 functions attenuates LTM while overexpression of NF1 enhances memory. Thus, memory strength is proportional to the amount of NF1 expressed. Third, NF1's functions within octopamine neurons are involved in supporting long-term memory. Silencing NF1 functions targeted to this group of neurons impairs memory. Manipulation of neuronal activity within this subgroup of neurons impact critically on memory strength within a time window after memory consolidation has been completed. Although these findings favor our hypothesis, we still cannot conclude that NF1 mediates retrieval of long-term memory. More investigation is needed to complete the proposed study and to make conclusion on role of NF1 in memory retrieval.

15. SUBJECT TERMS
neurofibromin, NF1, memory retrieval, long-term memory, Drosophila

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Introduction

This grant is funded for period of 2010-2013. We have requested a one-year extension. The proposed research investigates involvement of neurofibromatosis type 1 gene-encoded neurofibromin (NF1) in retrieval of long-term memory (LTM). The NF1 gene encodes a Ras-GAP that regulates not only Ras activity but also adenylyl cyclase activity (Guo et al., 1997). The mutations of this gene lead to the most common monogenic disorder, neurofibromatosis type 1. The critical role of NF1 in learning and memory has been indicated in human patients (North, 2000), as well as in mouse (Costa et al., 2001) and Drosophila (Guo et al., 2000) NF1 models. In *Drosophila*, we have revealed that NF1-regulated heterotrimeric G protein-dependent activation of the cAMP pathway is involved in learning while the NF1-regulated Ras activity is essential for LTM (Ho et al., 2007).

In this proposal, we hypothesize that NF1 is specifically involved in mediating memory retrieval, but not required for induction and consolidation of LTM. For this purpose, three specific aims are proposed, including (1) to determine NF1's role in memory retrieval; (2) to identify ligands that activate NF1 for memory retrieval; and (3) to locate the brain region at which NF1-dependent memory retrieval occurs. We have made progress towards an understanding of roles of NF1 in long-term memory, but have yet to complete this study for publication. Below, I will summary our research during the entire funding period.

Key words: neurofibromin, NF1, memory retrieval, long-term memory, Drosophila

Overall Project Summary

During the first funding year of this grant (2010-2011), we encountered difficulty in replicating memory phenotypes reported in the preliminary observations of this proposal, largely due to personnel changes—departure of a senor graduate student and taking over the project by a freshman graduate student who had significant gap in time with the senior student. With extensive efforts, we will be able to confirm all memory phenotypes and also made significant progress towards testing our hypothesis, but the difficulties drastically slowed down our progress to an extent that we have yet to complete our study for publications. Below, I will summarize progress made during last three years.

1. Specific long-term appetitive memory defects

After reestablishing isogenic genetic background for all mutants and controls used in our studies, we finally were able to confirm the learning defect and memory defect in NF1 mutants assayed through a well-established aversive classic conditioning paradigm. We then went on to assay the effects of NF1 mutations on an appetitive classic conditioning paradigm, which associate food with odor in starved flies. In contrast to aversive conditioning, we found that 24hr

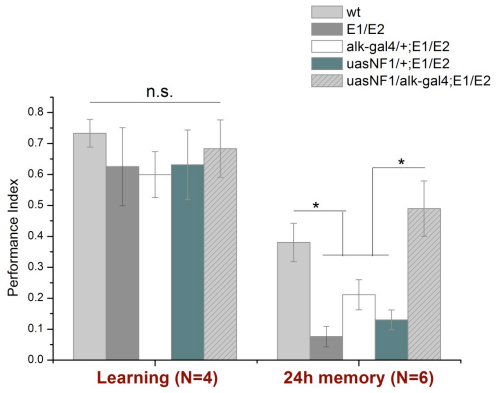

Fig. 1. Alk(38)-Gal4 expression of UAS-Nf1 in Nf1E1/E2 point mutants rescues 24h memory. For excluding effects of accumulated unknown genetic factors, heteroallelic Nf1E1/E2 mutant flies were used. E1/E2 exhibits significant 24h memory defects (*p<0.01) from parental lines, and Alk/uas-NF1; E1/E2 restores 24h memory performance.

long-term

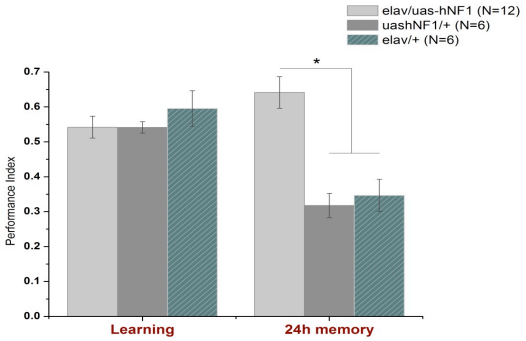

Fig. 2. overexpression of hNf1 enhances 24h appetitive memory. Flies with pan-neuronal overexpression of hNF1 (elav/+;hNF1 and elav/+;MBgal80/hNF1) show significant higher 24h memory performance (P<0.01). n as indicated.

memory was defective while immediate memory of appetitive conditioning remains similar as in controls (Fig. 1). This defect can be rescued by pan-neuronal expression of NF1 (Fig. 2). Moreover, pan-neuronal overexpression of the NF1 gene in the control background led to enhanced 24hr memory (Fig. 3). Thus, we confirmed that NF1 plays a critical role in both aversive and appetitive long-term memory. Since learning is close to normal, it would be advantageous to study NF1 with appetitive conditioning for simplifying interpretation.

2. Mapping of NF1 function in LTM

The enhanced appetitive LTM enables us performing behavioral screening of Gal4 lines for determining brain regions within which NF1 functions for LTM

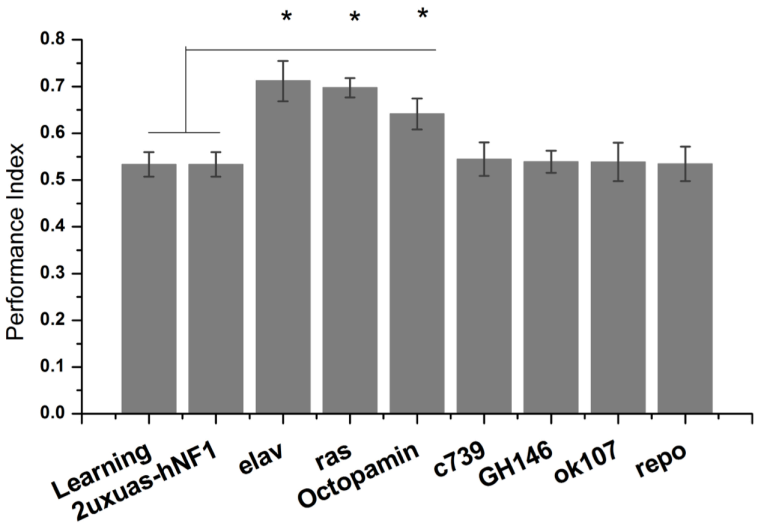

Fig. 3. Overexpression screen. Transgenic flies overexpressing NF1 with pan-neuronal driver Elav, and Ras2 as well as octopamine gal4 Tdc-2 showed enhanced 24h memory. Mushroom-body gal4 drivers 0k107 and c739, glia driver repo, and projection neuron driver GH146 did not. (ANOVA, p<0.05). n≥4.

because it only requires simple genetic manipulations. We were surprised to find that overexpression of the NF1 gene within mushroom body, a brain region critical for olfactory memory, didn't affect LTM, whereas overexpression within octopamine neurons showed a phenotype of enhanced appetitive LTM (Fig. 3).

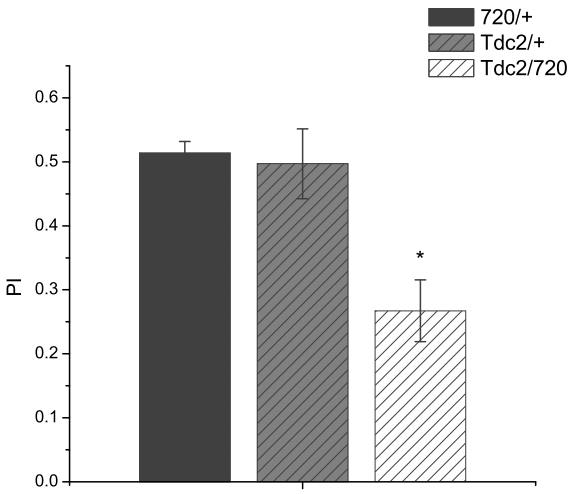

Fig. 4. Selectively knocking down NF1 in octopamine neurons attenuates appetitive LTM. Memory was tested at 24 hours after appetitive training. The NF1 RNAi line driven by octopamine GAL4 Tdc2 has significant lower LTM performance compared to the parental controls ($p < 0.05$). n = 8 for all groups.means ± SEM.

To confirm octopamine is indeed involved in NF1-dependent LTM, we showed that expression of NF1 within two Gal4 lines that target on enzymes required for synthesize octopamine dTdc2 and NP7088. In both case, the NF1 mutant memory defect was rescued. Conversely, expression of RNAi to silence NF1 within these neurons led to a LTM defect similar to that observed in NF1 mutants (Fig. 4).

3. Function of octopamine neurons

The ultimate goal of this proposal aims to investigate role of NF1 in retrieval of LTM. We now at least identified a subset of neurons within which functions of NF1 appear to be critical for appetitive LTM. Within this group of neurons, acutely induced expression of NF1 didn't work well enough to allow us to define the temporal window within which NF1 is required for supporting appetitive LTM.

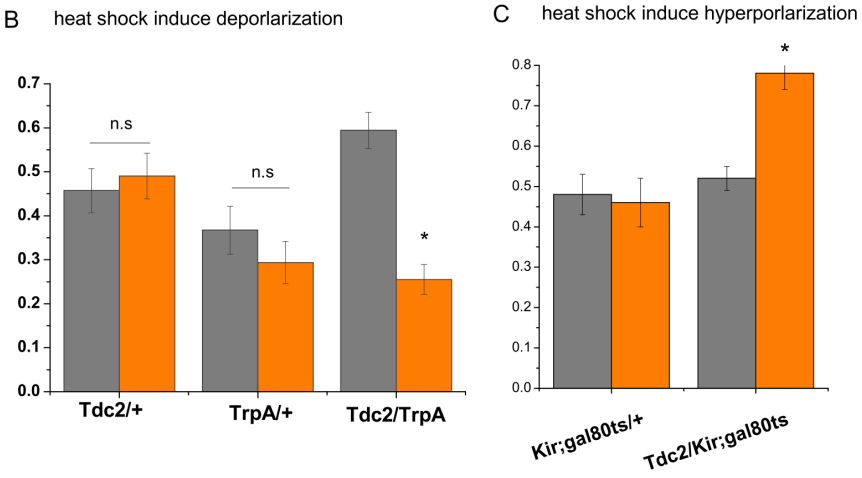

Fig. 5. Effects of activity in octopamine neurons on LTM. Flies were maintained at 18°C and heat shocked for a 12hrs duration12hrs after training. Memory was tested 48hrs after training. B. depolarizing octopamine neurons with TrpA channels significantly attenuated LTM performance. C. Hyperpolarizing octopamine neurons with expression of K channels enhanced memory compared to that of parental controls. n = 8 for both groups. means ± SEM.

On one side, we are still working to improve genetic tools for temporal controlled acute induction of gene expression. On the other hand, we examined effects of manipulating neuronal activity of octopamine neurons.

We found that either inactivation or activation of octopamine neurons immediately after training or during consolidation (first 12hr) had no effects on appetitive LTM, whereas in a later stage, inactivation through expression of 2.1 K+ channels led to enhanced LTM while activation through heat-shock that opens temperature-sensitive TrpA channels expressed within targeted octopamine neurons let to reduced LTM (Fig. 5), suggesting these neurons are involved in either maintenance or retrieval of LTM.

Key Research Accomplishments

1. We showed that NF1 not only impairs aversive LTM but also appetitive LTM. Appetitive LTM is more suitable for studying the role of NF1 in retrieval of LTM because appetitive learning and short-term memory are normal in NF1 mutant alleles.
2. We found that NF1 can bi-directionally regulate long-term memory, i.e. the loss of NF1 function attenuates LTM while overexpression of NF1 enhances LTM.
3. Functions of NF1 within octopamine neurons are critical for supporting LTM.

Conclusion

We made progress towards the goal of this proposal in revealing a role for NF1 in retrieval of long-term memory. Our new data favors the hypothesis. However, we need more time to complete our investigation and reach a publishable conclusion.

Publications, Abstracts, Presentations

None

Inventions, Patents, Licenses

None

Reportable Outcomes

None

Other Achievements

Publications

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Appendices

None

List of Personnel

Yi Zhong

Jennifer Beshel

Chunsu Xu